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Original research paper

# Simplex lattice design for optimization of the mass ratio of *Curcuma longa* L., *Curcuma zedoaria* (Christm.) Roscoe and *Curcuma aromatica* Salisb. to maximize curcuminoids content and antioxidant activity

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The aim of this work was to optimize the mass ratio of three *Curcuma* plants' rhizomes to obtain the highest curcuminoid content and antioxidant activity using the simplex lattice design. The selected *Curcuma* plants were *C. longa*, *C. zedoaria* and *C. aromatica*. The simplex lattice design was applied in the work. The individual curcuminoids (curcumin, demethoxycurcumin and bis-demethoxycurcumin) and total curcuminoid content were determined using high-performance liquid chromatography. *Curcuma longa* alone provided the highest content of bis-demethoxycurcumin and demethoxycurcumin. A mixture of *C. longa* and *C. aromatica* in the mass ratio of 72:28 % provided the highest curcumin content. The results showed that *C. longa* alone exhibited the highest antioxidant activity.

*Keywords*: *Curcuma*, curcuminoids, HPLC, simplex lattice design, optimization, antioxidant activity

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*Curcuma longa* L., *Curcuma zedoaria* (Christm.) Roscoe and *Curcuma aromatica* Salisb. are plants belonging to the Zingiberaceae family. The most extensively used parts of these three plants are the rhizomes. According to the *Thai Herbal Pharmacopoeia* (1), *C. longa* is used as stomachic, carminative, coloring agent and adstringent, whereas *C. zedoaria* is used as stomachic, antidiarrheal and emmenagogue. *C. longa* has several biological and pharmacological activities such as antioxidant, antiinflammatory, antiangiogenic and antiapoptotic (2). Additionally, it also exhibits antihyperlipidemic, anti-Alzheimer, antidepressant, antidiabetic, radioprotective and antimicrobial activities (3). *C. aromatica* exhibits antiinflammatory, wound healing, antitumor, anticancer, mosquito repellent, antiplatelet, antitussive, antioxidant, antimelanogenic and antinephrotoxic activities (4).

All three plants exhibit antioxidant activity, that can protect the skin. Curcuminoids, including curcumin (CUR), demethoxycurcumin (DMC), and bis-demethoxycurcumin (BDMC), are the major chemical constituents in their rhizomes (5). Among these three curcuminoids, CUR is the most abundant and its mechanism of antioxidant activity has been described (6, 7). CUR can defend biomembranes from peroxidative damage by scav-

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enging the reactive free radicals. CUR can degrade into several compounds, *i.e.*, *trans*-6(4'--hydroxy-3'-methoxyphenyl)-2,4-dioxo-5-hexanal, ferulic aldehyde, ferulic acid, ferruloyl methane and vanillin (8). Ferulic acid and vanillin exhibit antioxidant activity as well (9). Another work has also proven that CUR reveals a good antioxidant activity in several *in vitro* assays (10). In addition, DMC and BDMC also exhibit antioxidant activity (11).

The authors attempt is to distinguish the effect of the mass ratio of *C. longa*, *C. zedoaria* and *C. aromatica* rhizome powder on the content of curcuminoids and antioxidant activity, to further develop as an ingredient in the skincare formulations, using the simplex lattice design.

## EXPERIMENTAL

### *Materials*

Three standard curcuminoids (purity ≥ 99%), *i.e.*, BDMC, DMC and CUR were purchased from Chengdu Biopurify Phytochemicals Ltd., China. DPPH (2,2-diphenyl-1-picrylhydrazyl) was purchased from Sigma-Aldrich Inc., USA. Absolute ethanol was purchased from QRëC, New Zealand. Acetonitrile (HPLC grade) was purchased from Honeywell-Burdick & Jackson, USA. Acetic acid (AR grade) was purchased from Carlo-Erba, France.

### *Plant samples and extraction procedure*

The rhizomes of *C. longa*, *C. zedoaria* and *C. aromatica* were collected from Buached District, Surin Province, Thailand. *C. longa* and *C. aromatica* were collected in November 2017, while *C. zedoaria* was collected in January 2018. The voucher specimens CM-

*Table I. Coded and actual value of the model conditions obtained from simplex lattice design*

| Condition | Coded value | | | Actual value (mass of dried rhizome powder, g) | | |
|---|---|---|---|---|---|---|
| | $x_1$ | $x_2$ | $x_3$ | *C. longa* | *C. zedoaria* | *C. aromatica* |
| 1 | 1 | 0 | 0 | 30 | 0 | 0 |
| 2 | 0 | 1 | 0 | 0 | 30 | 0 |
| 3 | 0 | 0 | 1 | 0 | 0 | 30 |
| 4 | 0.5 | 0.5 | 0 | 15 | 15 | 0 |
| 5 | 0.5 | 0 | 0.5 | 15 | 0 | 15 |
| 6 | 0 | 0.5 | 0.5 | 0 | 15 | 15 |
| 7 | 0.67 | 0.17 | 0.17 | 20 | 5 | 5 |
| 8 | 0.17 | 0.67 | 0.17 | 5 | 20 | 5 |
| 9 | 0.17 | 0.17 | 0.67 | 5 | 5 | 20 |
| 10 | 0.33 | 0.33 | 0.33 | 10 | 10 | 10 |
| 11 | 0.33 | 0.33 | 0.33 | 10 | 10 | 10 |
| 12 | 0.33 | 0.33 | 0.33 | 10 | 10 | 10 |

CL001-1-11-2017, CM-CZ001-1-01-2018, and CM-CA002-2-11-2017 were coded for *C. longa*, *C. zedoaria* and *C. aromatica*, resp. (Drug and Herbal Product Research and Development Center, College of Pharmacy, Rangsit University).

Rhizomes of each plant were cleaned, sliced and sun-dried for 3 days. The dried rhizomes were pulverized and passed through a 0.841-mm (20-mesh) sieve. The 30 g of rhizome powder was extracted in an ultrasonic bath for 20 min at different mass ratios (Table I) using 100 mL of absolute ethanol. The extract was separated from the marc after vacuum filtration. The extraction was performed in triplicate; three filtrates were pooled and the solvent was removed by a rotary evaporator.

### *High-performance liquid chromatography (HPLC) conditions*

HPLC method, which was already reported, has been adapted and preliminarily validated (13, 19). Agilent 1260 Infinity HPLC instrument (Agilent Technologies, USA) was used in the analysis. ACE Generix column, 150×4.6 mm, 5 μm (Advanced Chromatography Technologies Ltd, UK) was used. An isocratic system composed of acetonitrile/acetic acid (1 %) in a volume ratio of 55:45, was used at a flow rate of 1 mL $min^{-1}$. The temperature of the column was set at 30 °C. The injection volume was 10 μL. The response was detected at 425 nm by a photodiode array detector.

### *HPLC method preliminary validation*

HPLC method validation was done according to ICH guidelines (12). The five topics, including linearity and range, selectivity, the limit of detection (*LOD*) and limit of quantitation (*LOQ*), precision and accuracy were demonstrated.

*Linearity and range.* – A stock solution of each BDMC, DMC and CUR in the concentration of 1000 μg $mL^{-1}$ was prepared. Methanol was used as the solvent. The mixtures of three standard curcuminoids for each standard were prepared from the stock solutions at five concentration levels (*i.e.*, 10, 25, 50, 75, and 100 μg $mL^{-1}$). Two sets of mixtures of three standard curcuminoids were prepared. They were filtered using a nylon syringe filter and injected into the HPLC instrument. Each concentration was analyzed in triplicate. The calibration curves for each BDMC, DMC and CUR were constructed. The linear equation, coefficient of determination ($R^2$), and range were reported.

*Selectivity.* – Selectivity was evaluated based on the selectivity ratio and chromatographic resolution. The selectivity ratio was calculated from the capacity factor values of the two peaks.

LOD *and* LOQ. – The standard solution was diluted with methanol. The *LOD* and *LOQ* were estimated based on the signal-to-noise ratio of 10:1 and 3:1, resp.

*Precision*. – Three concentration levels of each standard curcuminoid (25, 50, and 75 μg $mL^{-1}$) were analyzed. The analysis, done within-a-day and on three days, was used to calculate the percent relative standard deviation (RSD). These data were reported as intra-day and inter-day precision, resp.

*Accuracy.* – The individual three concentration levels of each standard curcuminoids (25, 50 and 75 μg $mL^{-1}$) were added to the extract (condition 10) solution. Each concentration level was analyzed in triplicate and the accuracy of each concentration level was reported.

### *Analysis of curcuminoids content*

The plant extract was diluted with methanol to a concentration of 200–500 μg $mL^{-1}$. It was filtered using a nylon syringe filter and injected into the HPLC instrument. Each sample was prepared in two sets ($n = 2$) and each set was evaluated in triplicate. The content of each curcuminoid was calculated from the calibration curve and total curcuminoid content was obtained from the summation of three curcuminoids.

### *Determination of antioxidant activity*

Antioxidant activity was determined using the DPPH radical scavenging assay. Extract volumes of 50–1000 μg $mL^{-1}$ were prepared using absolute ethanol as diluent. CUR, DMC, and BDMC were also tested, with a concentration range of 1–1000 μg $mL^{-1}$. The sample (100 μL) was pipetted into a 96-well plate ($n = 3$). 100 μL aliquot of the 200 μmol $L^{-1}$ DPPH ethanolic solution was then added to each well. They were incubated for 30 min in the dark at room temperature. The absorbance of the test sample was measured at 517 nm using a microplate reader (Benchmark Plus, Bio-Rad Laboratories, USA). An equal volume of absolute ethanol and DPPH ethanolic solution was used as a control. The half-maximal inhibitory concentration $IC_{50}$ was reported.

### *Optimization*

A simplex lattice design was used in this work. Design-Expert® version 11 was used for the optimization process. Three factors, *i.e.*, the mass ratios of *C. longa*, *C. zedoaria*, and *C. aromatica* rhizome powder were coded as $x_1$, $x_2$, and $x_3$, resp. The value of each factor was between 0 and 100 % and the summation of all factors equals 100 %. Five responses, *i.e.*, BDMC content ($y_1$), DMC content ($y_2$), CUR content ($y_3$), total curcuminoid content ($y_4$), and $IC_{50}$ value obtained from DPPH radical scavenging assay ($y_5$) were determined. The contour plot of each response was created. The plots between predicted values *vs.* actual values and residual *vs.* run were produced to confirm the stability and reliability of the estimation. The statistical data, *p*-value of the model and a lack of fit of each response were reported. The desirability function was used to select the optimum conditions – the optimum mass ratio of three *Curcuma* rhizome powders provided the highest curcuminoid content and maximal antioxidant activity (or minimal $IC_{50}$ value).

## RESULTS AND DISCUSSION

The method for analysis of curcuminoids in the rhizome of *C. longa*, *C. zedoaria*, and *C. aromatica*, and their mixtures was preliminary validated. The linearity parameters (linear equation and $R^2$), range and *LOD*, together with precision and accuracy data, are given in Table II. RSD values of intra- and inter-day precision were 0.1–0.2 and 0.2–3.6 %, resp. In addition, accuracy was 93.4–99.8 % for BDMC, 96.9–102.2 % for DMC, and 99.0–99.2 % for CUR. Fig. 1 shows that BDMC, DMC and CUR were eluted at the retention times of 3.95, 4.30 and 4.70 min, resp. The selectivity ratio of DMC *vs.* BDMC and CUR *vs.* DMC was 1.11 and 1.14, resp., however, for CUR/BDMC it was 1.27. All validation data indicated the acceptable analytical performances of the method.

*Table II. Analytical performances of HPLC method*

| Linear regression parameter | | | |
|---|---|---|---|
| | Analyte | | |
| | BDMC | DMC | CUR |
| Range (μg mL$^{-1}$) | 10–100 | 10–100 | 10–100 |
| Linear equation | $y = 161525x - 50686$ | $y = 185753x + 15028$ | $y = 226712x + 63963$ |
| $R^2$ | 0.9992 | 0.9995 | 0.9993 |
| *LOD* (ng mL$^{-1}$) | 2.5 | 5.0 | 5.0 |

| Precision and accuracy | | | | | |
|---|---|---|---|---|---|
| Analyte | Concentration (μg mL$^{-1}$) | Precision (RSD, %) | | Added concentration (μg mL$^{-1}$) | Accuracy (%) |
| | | Intra-day | Inter-day | | |
| BDMC | 25 | 0.2 | 2.2 | 25 | 93.4 |
| | 50 | 0.2 | 0.2 | 50 | 99.8 |
| | 75 | 0.2 | 0.2 | 75 | 98.5 |
| DMC | 25 | 0.2 | 3.6 | 25 | 96.9 |
| | 50 | 0.1 | 1.6 | 50 | 97.8 |
| | 75 | 0.1 | 1.2 | 75 | 102.2 |
| CUR | 25 | 0.1 | 0.7 | 25 | 99.2 |
| | 50 | 0.1 | 2.3 | 50 | 99.0 |
| | 75 | 0.1 | 2.5 | 75 | 99.2 |

Chromatographic parameters of the HPLC method are given in Table III.

Both individual and total curcuminoid content in rhizome powders mixture were estimated to observe their variation when different mass ratios of *C. longa*, *C. zedoaria*, and *C. aromatica* were applied. In this case, curcuminoids present in the extract were analyzed and calculated as curcuminoid content in the raw plant material (dried powder of rhizome). Fig. 1 shows the HPLC chromatogram of standard curcuminoids and extracts obtained under the same conditions as shown in Table I. *C. longa* alone (condition 1) mostly contained CUR followed by BDMC and DMC, resp. (Fig. 1a). It contained 1.06 % CUR, 0.63 % DMC and 0.91 % BDMC; total curcuminoids content roughly 2.6 %. Jayaprakasha *et al*. (14) showed *C. longa* to be composed of 1.01–5.65 % CUR, 0.83–3.36 % DMC and 0.42–2.16 % BDMC. In addition, total curcuminoid content was 2.34–9.18 % (14). The slight difference in the content of the curcuminoids was reported by Ali *et al*. (15) who stated 2.1 % CUR, 0.46 % DMC and 0.1 % BDM, whereas Osorio-Tobón *et al*. (16) showed 10.2 % CUR, 5.9–7.0 % DMC and 2.1–6.1 % BDMC in *C. longa*. *C. longa* powder filled in hard gelatin capsule contained high curcuminoids content as previously reported: 6.61–7.67 % CUR, 2.76–3.33 % DMC and 2.64–3.47 % BDMC (17).

*C. zedoaria* (condition 2) and *C. aromatica* (condition 3) mostly contained DMC (Figs. 1b,c). *C. zedoaria* contained 0.23 % DMC, 0.06 % BDMC and 0.04 % CUR (total curcuminoids

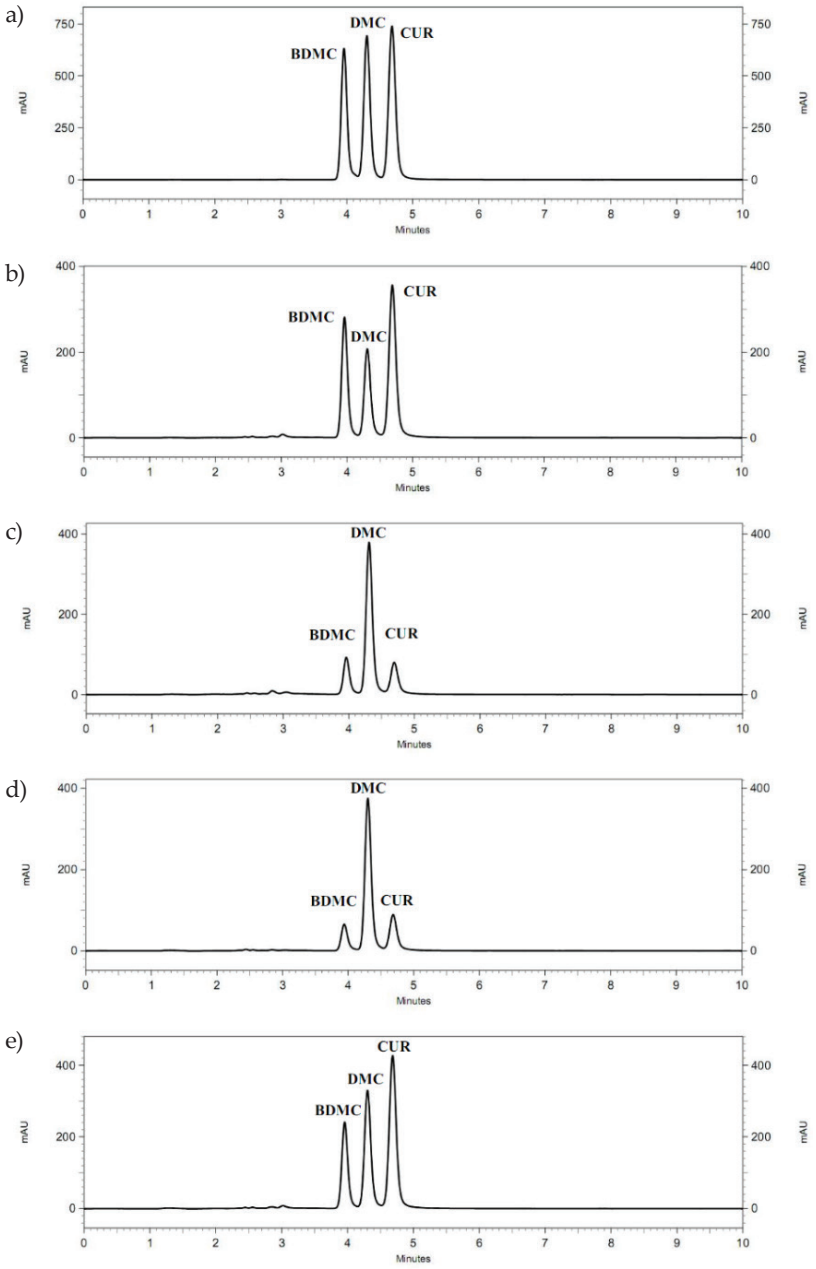


Fig. 1. HPLC chromatograms of: a) mixed standard curcuminoids (50 μg mL$^{-1}$) and extract obtained from: b) condition 1 (200 μg mL$^{-1}$), c) condition 2 (500 μg mL$^{-1}$), d) condition 3 (500 μg mL$^{-1}$) and e) condition 10 (500 μg mL$^{-1}$).

*Table III. Chromatographic parameters of HPLC method*

| Chromatographic parameter | Analyte | | |
|---|---|---|---|
| | BDMC | DMC | CUR |
| $t_R$ (min)[a] | 3.95 ± 0.01 | $4.30 \pm 0.00_1$ | $4.70 \pm 0.00_3$ |
| Capacity factor ($k'$)[b] | 2.07 ± 0.06 | $2.30 \pm 0.00_2$ | 2.63 ± 0.06 |
| Tailing factor ($T_f$)[b] | 1.20 ± 0.17 | 1.37 ± 0.15 | 1.23 ± 0.25 |
| Number of theoretical plates ($N$)[b] | 2196 ± 94 | 2252 ± 255 | 2369 ± 74 |
| Resolution ($R_s$)[b] | 1.50 ± 0.26 | 1.50 ± 0.26 | 1.53 ± 0.25 |
| Selectivity ($\alpha$)[b] | 1.11 ± 0.03 | | 1.14 ± 0.03 |
| | – | 1.11 ± 0.03 | 1.27 ± 0.03 |

Mean ± SD. [a] $n = 3$, [b] $n = 6$.

0.3 %). *C. aromatica* contained 0.43 % DMC, 0.09 % BDMC and 0.08 % CUR (total curcuminoids content 0.6 %), with two times higher total curcuminoids compared with *C. zedoaria*, whereas *C. longa* had approx. 8 and 4 times, resp., higher content of total curcuminoids than the other two species. Paramapojn and Gritsanapan (18) reported that ethanolic extract of *C. zedoaria* rhizome contained 2.73 % CUR, 7.37 % DMC and 1.40 % BDMC, which was close to our results: 1.02 % CUR, 5.98 % DMC and 1.63 % BDMC. Other work of the same colleagues showed that 0.50–0.73 % CUR, 0.23–1.43 % DMC, 0.12–0.44 % BDMC were found in *C. zedoaria* powder (19). *C. zedoaria* consisted of 0–1.5 % CUR as reported by Morishita *et al.* (20). However, no CUR, DMC and BDMC in *C. zedoaria* methanolic extract were reported in a publication by Tohda *et al.* (21) but 0.11 % CUR with no DMC and no BDMC in *C. aromatica* methanolic extract. Morishita *et al.* (20) reported that *C. aromatica* contained 0.05–0.1 % CUR estimated on a dry mass basis. However, sometimes *C. aromatica* may contain more CUR than DMC (22, 23). Fig. 1e shows the mixture of three plants rhizome powders in equal mass ratios (condition 10) which mostly had CUR followed by DMC and BDMC.

Our work documented that CUR exhibited the most potent antioxidant activity compared to DMC and BDMC: $IC_{50}$ values of CUR, DMC and BDMC were 38, 97, and 1,375 μmol $L^{-1}$ (namely, 17.83 ± 1.59, 32.73 ± 5.07 and 424.10 ± 20.40 μg $mL^{-1}$), resp. All three curcuminoids showed good antioxidant activity. The presence of the methoxy group in CUR could increase antioxidant activity (9), so CUR showed the best antioxidant activity compared with DMC and BDMC. The $IC_{50}$ value of the standard curcuminoids varied in the literature. Abas *et al.* (24) reported the $IC_{50}$ values of CUR, DMC, and BDMC of 31.8, 92.5, and 104.4 μmol $L^{-1}$ (or 11.7, 31.3, and 32.2 μg $mL^{-1}$) (24) with a largely different $IC_{50}$ value of BDMC compared with our result. The low $IC_{50}$ values were reported for CUR by Borra *et al.* (25) of 2.9 μmol $L^{-1}$ (or 1.1 μg $mL^{-1}$) and Sökmen and Khan (26) of 22.8 μmol $L^{-1}$ (or 8.4 μg $mL^{-1}$). On the other hand, high $IC_{50}$ values were also reported: Ak and Gulcin (10) reported that CUR had $IC_{50}$ of 94.6 μmol $L^{-1}$ (or 34.9 μg $mL^{-1}$).

Regarding the plant extracts, extract obtained from the condition 1, which contained *C. longa* alone, exhibited the lowest $IC_{50}$ value (33.83 ± 14.05 μg $mL^{-1}$, equivalent to 26.01 ± 1.24 μg $mL^{-1}$ total curcuminoids), indicating the most potent antioxidant activity. The $IC_{50}$ of *C. longa* extract was similar to the results of the previous work (33.5 μg $mL^{-1}$) (27). However, low $IC_{50}$ of *C. longa* extract of 9.7 μg $mL^{-1}$ was reported by Zaeoung *et al.* (28). Further-

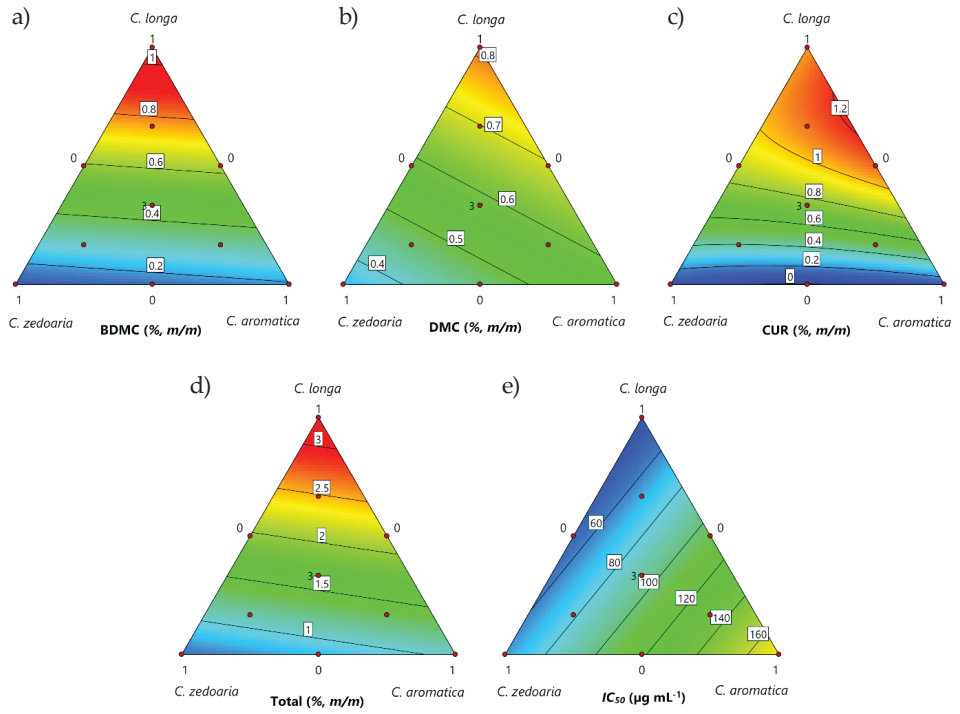


Fig. 2. Contour plots of the model conditions of: a) BDMC content, b) DMC content, c) CUR content, d) total curcuminoid content, in the plant powder mixtures, and e) $IC_{50}$ value of the extract obtained from the plant powder mixtures.

more, Tanvir *et al*. (29) reported that $IC_{50}$ values of *C. longa* aqueous and ethanolic extracts ranged 5.31–16.55 and 1.08–3.03 μg mL$^{-1}$, resp. In this work, we found that *C. zedoaria* extract obtained from condition 2 and *C. aromatica* extract obtained from condition 3 had $IC_{50}$ values of 54.63 ± 3.37 and 138.72 ± 4.76 μg mL$^{-1}$, resp., which were equivalent to 8.63 ± 0.96 and 7.56 ± 0.17 μg mL$^{-1}$ total curcuminoids, resp. The other samples (extracts obtained from conditions 4–12) contained three plants in different ratios, $IC_{50}$ values ranged from approximately 40 to 200 μg mL$^{-1}$. Our result was different from the previous reports. Souria *et al*. (30) showed that *C. zedoaria* extract had an $IC_{50}$ value of 10.60 μg mL$^{-1}$, whereas Nahak and Sahu (31) reported 40 μg mL$^{-1}$. They also reported an $IC_{50}$ value of *C. aromatica* extract of 120 μg mL$^{-1}$, which was close to the result of Srividya *et al*. (32) of 132.5 μg mL$^{-1}$.

Fig. 2 shows the contour plots of the model conditions of curcuminoids content in dried rhizome powder and $IC_{50}$ value of the extract. The maximum BDMC, DMC and total curcuminoid content in dried rhizome powder were found when *C. longa* was used alone, whereas the minimum was found when *C. zedoaria* was used alone. In the case of CUR in rhizome powder, the maximum value was achieved when *C. longa* was mixed with *C. aromatica* in the mass ratio of 72:28, while the minimum value was found when *C. zedoaria* was mixed with *C. aromatica* in the mass ratio of 89:11. The maximum $IC_{50}$ value of the extract

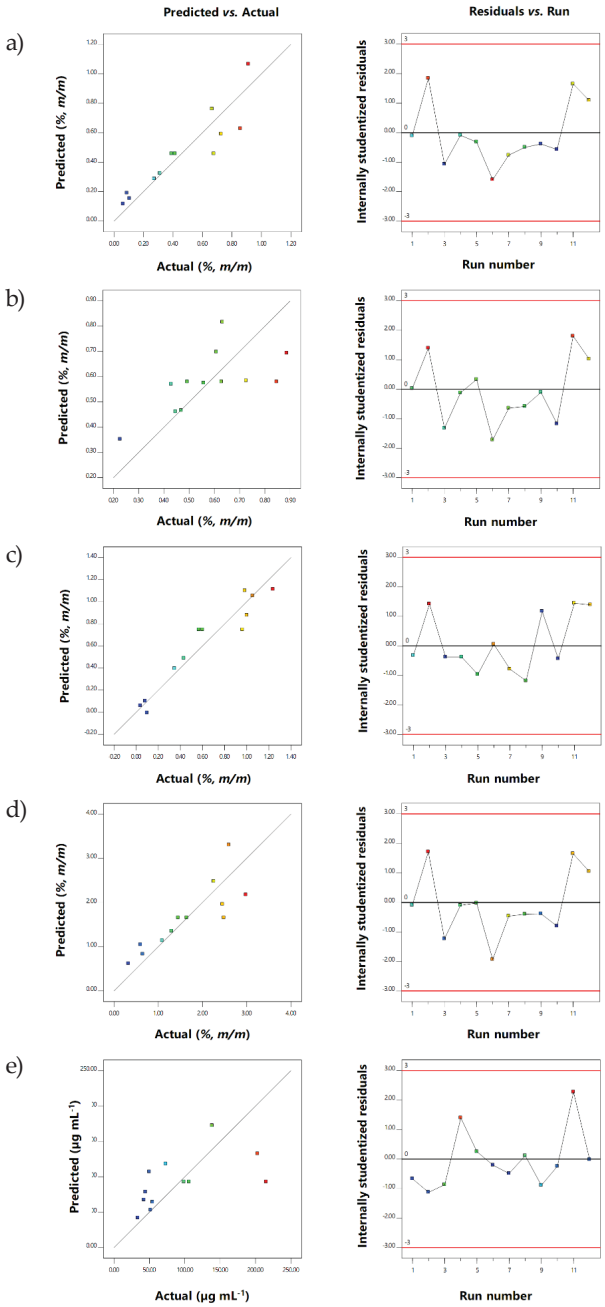


Fig. 3. Predicted *vs.* actual plots (left) and residual *vs.* run plots (right) of: a) BDMC content, b) DMC content, c) CUR content, d) total curcuminoids content, and e) $IC_{50}$ value.

was found when *C. aromatica* was used alone and the minimum $IC_{50}$ value when *C. longa* was used alone. The results indicated that the most potent antioxidant activity was found when *C. longa* was used alone.

The predicted values obtained from the Design-Expert® were based on the equations shown below. Fig. 3 shows the predicted *vs.* actual value plots and the internally Studentized residuals *vs.* run number plots of the model condition of BDMC, DMC, CUR and total curcuminoids content in rhizome powder and $IC_{50}$ value of the extract. The $R^2$ between the predicted and actual values of model conditions of BDMC, DMC, CUR and total curcuminoids content in rhizome powder, and $IC_{50}$ value of the extract were 0.8265, 0.4265, 0.9183, 0.7136 and 0.3392, resp. The moderate to high correlation was found for BDMC, CUR and total curcuminoid content, whereas low correlation was found in DMC content and $IC_{50}$ value. The internally Studentized residuals *vs.* run number plots displayed that the data was within the red borderline, indicating data distribution within a 95 % confidence interval (CI). These results could assure the stability of the prediction. The data in Fig. 3 could approve the reliability and stability of the prediction by computer software (33, 34). Table IV shows the *p*-values of the model and the lack of fit of various responses. The *p*-values of experimental models of $y_1$, $y_3$, and $y_4$ were less than 0.05 indicating that model terms were significant, whereas the *p*-values of models of $y_2$ and $y_5$ were not significant. The lack of fit *p*-values of all responses was more than 0.05 implying that the lack of fit was not significant relative to the pure error. So the experimental design was not significantly affected by the error or coincidence.

*Table IV. Statistical data (p-value) of the model and lack of fit of each response*

| Response | *p*-value | |
|---|---|---|
| | Model | Lack of fit |
| $y_1$ | 0.0004* | 0.6970 |
| $y_2$ | 0.0820 | 0.7042 |
| $y_3$ | 0.0033* | 0.8333 |
| $y_4$ | 0.0036* | 0.6218 |
| $y_5$ | 0.1550 | 0.7096 |

* Statistical significance

*Table V. Predicted value, actual value, percent error, and 95% confidence interval of the prediction*

| Response | Predicted value | Actual value | Error (%) | 95 % CI (lower-upper) |
|---|---|---|---|---|
| $y_1$ (%, *m*/*m*) | 1.07 | 0.91±0.10 | –17.6 | 0.84–1.29 |
| $y_2$ (%, *m*/*m*) | 0.82 | 0.63±0.03 | –30.2 | 0.56–1.07 |
| $y_3$ (%, *m*/*m*) | 1.05 | 1.06±0.01 | 0.9 | 0.67–1.44 |
| $y_4$ (%, *m*/*m*) | 3.30 | 2.60±0.12 | –26.9 | 2.43–4.17 |
| $y_5$ (μg mL$^{-1}$) | 41.81 | 33.83±6.60 | –23.6 | 0.00–83.62 |

CI – confidence interval

The equations used for the estimation of each response are shown below:

$$y_1 = 1.07x_1 + 0.12x_2 + 0.19x_3$$

$$y_2 = 0.82x_1 + 0.35x_2 + 0.57x_3$$

$$y_3 = 1.05x_1 + 0.06x_2 + 0.10x_3 + 1.28x_1x_2 + 2.14x_1x_3 - 0.34x_2x_3$$

$$y_4 = 3.30x_1 + 0.62x_2 + 1.05x_3$$

$$y_5 = 41.81x_1 + 64.08x_2 + 172.34x_3$$

The desirability function could be used for selecting the optimum condition. In this case, the optimum condition was the optimum mass ratio of three *Curcuma* rhizome powders providing the highest curcuminoid content and the maximal antioxidant activity (or minimal $IC_{50}$ value). The desirability value equal to 1 should indicate a fully desired response (35). The desirability value, obtained from the computer software Design-Expert®, showed that the maximum desirability value of 0.978 was reached when *C. longa* was used alone. So, the optimum condition was approached when *C. longa* was used alone. The predicted value, actual value, percent error and range of 95 % CI of the prediction are shown in Table V. When the prediction value was compared with the actual value, the error of the prediction was found approximately 30 % – the lowest percent error was found in $y_3$ of 0.9 %, and the highest percent error was found in $y_2$ of –30.2 %. However, the actual value was within the lower and upper limit of the 95 % confidence interval, indicating that the data obtained from the computer software were moderately accurate.

## CONCLUSIONS

This work used simplex lattice design to optimize the mass ratio of three *Curcuma* plants' rhizomes to obtain the high curcuminoid content and antioxidant activity. Three plants, *i.e.*, *C. longa*, *C. zedoaria* and *C. aromatica*, were included in the work. The CUR was the major compound found in *C. longa*, whereas DMC was the major compound found in *C. zedoaria* and *C. aromatica*. *C. longa* alone gave the highest content of BDMC and DMC. *C. longa* mixed with *C. aromatica* gave the highest CUR content. The lowest $IC_{50}$ value, obtained from DPPH radical scavenging assay, was found when *C. longa* was used alone. From the optimization process, *C. longa* alone reveals the highest total curcuminoid content and the most potent antioxidant activity compared with *C. zedoaria*, *C. aromatica* and their admixtures. In conclusion, the simplex lattice design could be used to optimize the mass ratio of three *Curcuma* plants rhizomes to obtain the maximized individual and total curcuminoid content as well as antioxidant activity with moderate accuracy.

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